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Report

Tamoxifen and fenretinide in women with metastatic breast cancer

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Summary

Background: Tamoxifen and fenretinide combination therapy has been shown to be an active treatment regimen in metastatic breast cancer patients. This pilot study sought to determine whether the addition of fenretinide to tamoxifen would be associated with antitumor activity in metastatic breast cancer patients who had been previously treated with tamoxifen or who had hormone receptor negative disease. The effect of this therapy on circulating plasma transforming growth factor-beta (TGF-β) levels and serum lipids was also examined.

Patients and Methods: Thirty-one patients were treated with tamoxifen (20 mg po daily), and fenretinide (400 mg po daily with a 3-day drug holiday each month). Plasma TGF-β testing was performed using isoform specific sandwich ELISA.

Results: Twenty four of the 31 patients were evaluable for an antitumor response including 14 estrogen receptor (ER) positive patients who had failed prior tamoxifen therapy, seven ER-negative patients, and three hormone therapy naive ER-positive patients. There were no objective antitumor responses; three patients had stable disease for 8, 8, and 24 months. Five patients (16%) discontinued therapy for toxicity (one for grade 3 skin rash and four for abnormal dark adaptation). There was a statistically significant decrease in total cholesterol (median change per patient of -13.5 mg/dl; $p = 0.049$, a 6.5% decrease), and an increase in HDL levels (median change per patient of $+18$ mg/dl, $p = 0.0001$, a 35% increase) with tamoxifen and fenretinide therapy.

TGF-β1 plasma levels were normal in 26 of 28 patients, and no changes in these levels post-treatment were demonstrated.

Conclusions: Tamoxifen and fenretinide therapy is not an active combination in ER negative metastatic breast cancer or in patients whose disease has progressed on tamoxifen. This combination had a beneficial effect on total serum cholesterol and HDL levels with no associated rise in serum triglyceride levels. The 400 mg dose of fenretinide was associated with symptomatic nyctalopia in one-third of patients making it an unsuitable dose for use in breast cancer prevention studies.

Introduction

Tamoxifen is a potent selective estrogen receptor (ER) modulator with important treatment and preventive effects in breast cancer [1]. The retinoids are vitamin A derivatives which play important roles in growth, vision, reproduction, immune function, and differentiation [2, 3]. Fenretinide [N-(4-hydroxyphenyl) retinamide (4-HPR)], a synthetic derivative of retinoic acid, has been shown to exhibit significant antitumor activity in rat carcinogenesis models [4–6]. Combined treatment with tamoxifen and fenretinide has been shown to be superior to either agent alone in preventing neoplastic mammary lesions in the N-methyl-N-nitrosourea (NMU) rat carcinogenesis model [7, 8].

No objective tumor responses were observed in 15 metastatic breast cancer patients in a Phase II study of fenretinide (300–400 mg/day) as a single agent [9]. However, in a Phase I/II study of combined tamoxifen and fenretinide in previously untreated metastatic breast cancer patients, 10 of 12 patients experienced improvement or stabilization of disease [10].

The mechanisms by which tamoxifen and the retinoids exhibit their anti-tumor and preventive effects are not fully understood. Both agents have been shown to decrease plasma insulin-like growth factor-1 (IGF-1) levels which are involved in breast cancer growth through an autocrine/paracrine mechanism [11–14].

Transforming growth factor-beta (TGF-β), a family of growth inhibitory proteins, may inhibit breast epithelial cell growth through paracrine effects [15, 16].

Retinoids have been shown to increase TGF-β2 and β3 isoforms in multiple epithelia of vitamin deficient rats [17]. Tamoxifen has been shown to increase the extracellular expression of the TGF-β1 isoform in the stroma of human breast cancer lesions [18]. In addition, TGF-β has been shown to inhibit the growth of ER-negative breast cancer [16]. The combination of tamoxifen and fenretinide, therefore, may have additive or even synergistic anti-neoplastic effects by mechanisms that include the induction of multiple TGF-β isoforms and via the inhibition of both ER-positive and ER-negative breast cancer cells.

We have conducted a pilot trial of tamoxifen and fenretinide to determine the antitumor activity and toxicity of this combination in patients with hormone receptor negative and tamoxifen-pretreated metastatic breast cancer. We chose a fenretinide dose of 400 mg/day with a 3-day drug holiday every 4 weeks because this was the highest dose studied in a Phase I combination study which showed acceptable toxicity [10]. Studies of higher doses of fenretinide have been terminated because of nyctalopia (Fenretinide Investigator's Brochure, 1992). In addition, because of the interest in this combination as adjuvant therapy and for chemoprevention [19], we measured plasma levels of the TGF-β isoforms, and serum lipid levels before and after therapy.

Patients and methods

Eligibility

Patients entered on study had bidimensionally measurable metastatic breast cancer that was either ER positive and pretreated with tamoxifen, or ER and progesterone receptor (PR) negative and not pretreated with tamoxifen. Additional ER-positive patients who were not pretreated with tamoxifen were entered on study to evaluate their lipid profiles and circulating TGF-β levels pre- and post-therapy.

All patients met the following eligibility criteria: Karnofsky performance status >70; serum glutamic oxalacetic transaminase (SGOT), alkaline phosphatase, and total bilirubin $<2\times$ upper limit of normal unless there was evidence of hepatic tumor involvement; serum creatinine <1.7 mg/dl; absolute neutrophil count >1000 per cubic millimeter and platelet count >75 per cubic millimeter; at least 4 weeks must have elapsed since prior chemotherapy. All patients gave informed consent according to National Institutes of Health (NIH) Clinical Center Guidelines.

Evaluation

Pre-therapy evaluation included a complete history and physical examination, complete blood count with differential, platelet count, urinalysis, electrolytes, blood urea nitrogen, creatinine, liver panel, mineral panel, uric acid, lipid profile (total cholesterol, high density lipoproteins (HDL), triglycerides), baseline plasma TGF-β determination, pregnancy test for women of childbearing potential, and ophthalmologic exam. Staging studies included a chest X-ray, bone scan, chest and abdominal CT scans, and mammogram.

Physical examination and laboratory tests were repeated every 4 weeks, staging evaluations every 8 weeks, and plasma TGF-β determinations were repeated 1–3 months after the initiation of therapy. Serum lipid measurements were repeated 1–6 months after patients had begun therapy. Ophthalmologic exams including Goldmann–Weekers dark adaptometry were performed at baseline and during the first cycle of therapy. The Goldman–Weekers test involves preadapting the patient to bright light for 5 min, after which threshold measurements in complete darkness are taken. The final dark adapted threshold was determined for each patient tested. Initially, ophthalmologic exams were performed in conjunction with

the patients' 4 week clinic visits, a time that generally coincided with their drug holiday or the first week of therapy. After it was recognized that some patients were developing symptoms of impaired dark adaptation, efforts were made to test patients during the last few days of the 25-day cycle of fenretinide administration.

Treatment

Tamoxifen was administered 20 mg orally daily and fenretinide 400 mg orally days 1–25, with a drug holiday on days 26–28 of each 4 week cycle. Patients were instructed to take fenretinide with the largest meal of the day to enhance its absorption.

TGF-β analysis

TGF-β testing in the plasma was performed by acid ethanol extraction followed by isoform specific sandwich ELISA as previously described [20].

Response and toxicity criteria

A complete response was defined as total disappearance of all clinical evidence of disease on two determinations separated by at least 4 weeks. A partial response was defined as at least a 50% reduction in the volume of all measurable tumor on two determinations separated by at least 4 weeks. Progressive disease was defined as a greater than 25% increase in the volume of the measurable lesions. Patients who failed to meet the criteria for an objective response or progressive disease were considered to have stable disease. Patients were evaluable for response after completing two 28-day cycles of therapy. The NCI Common Toxicity Criteria were used to evaluate toxicities.

Statistical analysis

The Wilcoxon rank sum test was used in the evaluation of cholesterol and lipid determinations.

Results

Thirty one patients with measurable metastatic breast cancer were enrolled on study. Patient characteristics are shown in Table 1. A total of 124 cycles of therapy were administered and the median number of cycles administered per patient was 3 (range 1–24). The median age of the patients was 50 years. Nineteen patients had been previously treated with tamoxifen for their ER-positive disease (6 adjuvant, 13 metastatic). Six patients had ER- and PR-negative disease. Six had ER positive disease that had not been treated previously with tamoxifen. Patients had received a median of two prior chemotherapy regimens and had a median of two sites of metastatic disease.

Table 1. Patient characteristics, prior treatments and sites of disease

Patient characteristics	
Total number of patients	31
Mean age (range)	50 (29–74)
Median no. of prior chemotherapy regimens (range)	2 (1–7)
Median no. of prior hormonal therapy regimens (range)	1 (1–5)
Patients with ER+ breast cancer	25
previously treated with tamoxifen	
Adjuvant	6
Metastatic	13
None	6
Patients with ER/PR-breast cancer	6
Patients with no. of disease sites (%)	
1	5(16%)
2	19 (61%)
>3	7 (23%)
Patients with metastatic disease to	
Lung	14 (45%)
Liver	13 (42%)
Bone	22 (71%)
Soft tissue	13 (42%)

Toxicity

Table 2 illustrates the clinical toxicities (exclusive of ophthalmologic) observed in this study. Of the 31 patients, five (16%) discontinued therapy due to toxicity (four patients for difficulty with night vision, and one patient for a generalized grade 3 skin rash). There were two deaths on study that were considered unlikely to be related to therapy (one suicide, and one acute nonlymphocytic leukemia).

Sixteen of the 31 patients (52%) had ophthalmologic complaints while on study. Eleven patients reported difficulty with dark adaptation, and this symptom required discontinuation of therapy in four patients. Seven patients with mild symptoms were continued on therapy without worsening of their symptoms. Ten pa-

Table 2. Clinical toxicities (exclusive of ophthalmologic) graded using the NCI common toxicity criteria

Clinical toxicities (exclusive of ophthalmologic), $n = 31$ patients				
Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Rash	3	0	1	0
Nausea	4	0	0	0
Abdominal pain	0	1	0	0
Depression	1	0	0	0
Diarrhoea	0	1	0	0
Drylips	1	0	0	0
Fatigue	2	1	0	0
Hot flashes	13	1	0	0

tients reported mild intermittent ocular symptoms not related to dark adaptation, including itching, burning, or dry eyes (four patients), color distortion described as a yellow tinting of objects (four patients) or black spots (two patients), and blurred vision (one patient). All ocular toxicities reversed following discontinuation of therapy.

All patients had ophthalmologic exams performed at protocol entry; only one patient had a slightly abnormal dark adaptometry test prior to initiating therapy. Twenty patients also had follow-up ophthalmologic exams performed 1–3 months after initiating therapy (Table 3). Of these 20 patients, 15 (75%) had normal dark adaptometry exams. Five patients (25%) had an abnormal exam on at least one occasion. Six of the patients with normal exams complained of symptoms of nyctalopia which improved during the drug holiday. These patients were tested during the three-day drug holiday or early in the subsequent cycle of fenretinide therapy (days 1–8). It is possible that their dark adaptometry results may have been abnormal if performed during days 21–25 of fenretinide therapy. Indeed, five of the seven patients tested between cycle days 20–25 had abnormal dark adaptometry exams. Four of these five patients did have symptomatic nyctalopia.

Lipid studies

Twenty-two patients had baseline and follow-up lipid determinations, including total cholesterol, high density lipoprotein (HDL) and triglyceride analysis. The time from the baseline evaluation to the final determination (obtained while on therapy or within 1 week of completing therapy) ranged from 27 to 679 days, with a median of 63 days.

Table 3. Results of Goldman–Weekers (GW) dark adaptometry testing and patients' visual symptoms on study

No. of patients	Symptoms	Day of GW test	GW test result	Therapy stopped?
13	Yes	Holiday	Normal	Yes
29	Yes	Holiday	Normal	Yes
		21	Abnormal	
19	Yes	2	Normal	No
10	Yes	14	Normal	Yes
		23	Abnormal	
24	Yes	5	Normal	No
14	Yes	Holiday & 2	Normal	No
		Holiday & 25	Abnormal	
9	Yes	2	Normal	Yes
8	Yes	21	Abnormal	No
31	No	20	Abnormal	No
1	Yes	1&8	Normal	No
25	Yes	2	Normal	No
21	No	Holiday	Normal	No
2	No	1	Normal	No
16	No	1&2	Normal	No
5	No	21	Normal	No
3	No	Holiday	Normal	No
12	No	2&7	Normal	No
6	No	9	Normal	No
26	No	25	Normal	No
11	No	Holiday & 1	Normal	No

The median baseline total cholesterol level was 202 mg/dl, (range 143–260), and the median cholesterol level on therapy was 189 mg/dl, (range 146–265). This represents a median change of −13.5 mg/dl (range −6 to 51) (6.5%) which was marginally significant ($p = 0.049$) by the Wilcoxon signed rank test. The median baseline HDL level was 51 mg/dl (range 32–83), and the median HDL level on therapy was 69 mg/dl (range 40–102), a 35% increase. Except for one patient in which the HDL level decreased while on therapy, all other patients were noted to have an increase in serum HDL. Thus, the null hypothesis that the mean change was zero was rejected at the $p < 0.0001$ level by the Wilcoxon signed rank test. There was no significant increase or decrease in the patients' triglyceride levels with treatment.

TGF-β plasma levels

Twenty-eight patients had baseline plasma levels of TGF-β levels assayed before beginning tamoxifen and fenretinide therapy; of these patients 20 had plasma

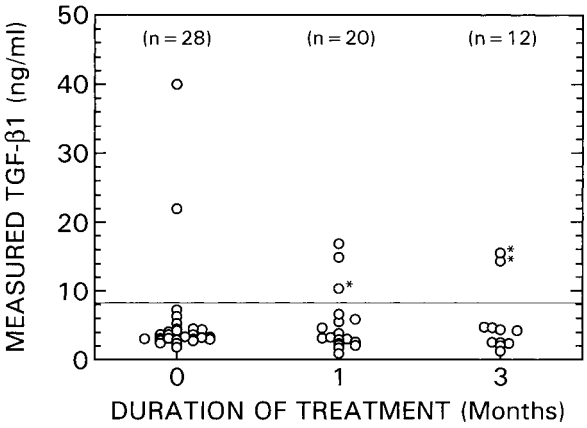

Figure 1. Measured plasma TGF-β levels in breast cancer patients before and after treatment with tamoxifen and 4-HPR. TGF-β1 plasma levels were determined at baseline (time 0) and after 1–3 months of combined tamoxifen and fenretinide therapy in 28 patients with metastatic breast cancer. The solid line indicates the upper limit of normal (2 standard deviations above mean value of 4.1 ± 2.0 ng/ml). Extensive platelet degranulation *ex vivo* causing elevated TGF-β1 levels (*).

levels measured after 1 month of treatment, and 12 patients after 3 months (Figure 1). TGF-β1 levels obtained prior to therapy were normal in 26 of 28 patients and in all but one case were unchanged after 1–3 months of therapy. Two patients had elevated TGF-β1 levels prior to therapy; one patient had extensive bone and liver disease, and a second patient had completed radiation therapy to her cervical spine 4 weeks prior to study entry. Only one patient with a normal TGF-β1 level at baseline demonstrated an increase in plasma TGF-β1 after 1 month of therapy.

Two patients had detectable, but low, plasma TGF-β2 levels (0.3–0.7 ng/ml). In the remaining patients tested, plasma TGF-β2 levels were below the detection limit of 0.2 ng/ml, and TGF-β3 levels were below 0.1 ng/ml in all patients tested. No increases in plasma TGF-β2 or TGF-β3 were detected post-therapy.

Antitumor activity

Of the 19 ER-positive patients who had received prior tamoxifen therapy, 16 patients were evaluable for response. Two patients were removed from study because of toxicity during the first two cycles, and one patient refused further therapy. Fourteen of the evaluable patients completed at least two cycles of therapy. No objective responses were observed in these patients who had been previously treated with tarnoxifen, although one patient had stable chest wall disease for more than 24 months with tamoxifen and fenretinide.

There were no objective responses in the six patients with ER-negative breast cancer, nor did any patient achieve prolonged stable disease. When 14 evaluable ER-positive, tamoxifen-pretreated and six ER/PR negative patients demonstrated no objective responses (nor stable disease in the latter group), accrual to these cohorts was stopped. Of the six patients who were ER positive and had not been treated with tamoxifen, three were evaluable for a response (one patient discontinued therapy due to toxicity, a second did not complete two cycles, and the other experienced a medication delay). Of these three patients, two had stable disease during 8 months of tamoxifen and fenretinide therapy.

Discussion

The low toxicity profiles of tamoxifen and fenretinide as single agents in human studies, and the synergistic effects of tamoxifen and retinoids in preventing mammary cancer in animal carcinogenesis models, make this combination attractive for study in both the treatment and prevention of breast cancer. In this study, the most frequent toxicity of combined tamoxifen (20 mg) and fenretinide (400 mg) therapy was ophthalmologic, with 16 of 31 patients (52%) developing ocular complaints. These symptoms were all reversible upon discontinuation of therapy both clinically and by dark adaptometry testing. Patients with mild symptoms of nyctalopia and normal dark adaptometry were able to continue therapy without worsening of their symptoms. Fenretinide treatment is known to be associated with nyctalopia [19]. The results of detailed ophthalmologic testing in patients participating in a secondary breast cancer prevention study of a lower dose of fenretinide, 200 mg daily with a 3-day drug ophthalmologic testing in patients participating in a secondary breast cancer prevention study of a lower dose of fenretinide, 200 mg daily with a 3-day drug holiday each month, have been previously reported [21]. In this study, 50% of patients on fenretinide demonstrated mild-to-moderate alterations of dark adaptometry as measured by the Goldmann–Weekers adaptometer; however, only half of these patients were symptomatic. We have previously demonstrated significant delays in the rod-cone break in 16 of 22 high risk women who were treated with tamoxifen 20 mg daily, and fenretinide 200 mg daily with a 3-day drug holiday each month in a chemoprevention trial [22]. Interestingly, however, only two of these 16 patients

reported subjective changes in dark adaptation. We believe the higher proportion of patients with symptomatic nyctalopia in our current study is due to the 400 mg fenretinide dose chosen for treating metastatic disease. This dose of fenretinide is unsuitable, therefore, for use in chemoprevention trials in women at risk for developing breast cancer.

Our study has demonstrated the importance of conducting dark adaptometry testing in symptomatic patients while they are taking fenretinide and preferably towards the end of the 25-day treatment period because the nyctalopia is rapidly reversible and most often disappears during the 3-day drug holiday. Evaluation early in the cycle or during the drug holiday may miss or underestimate the degree of dark adaptation impairment. Patients with significant symptoms of impaired dark adaptation should discontinue fenretinide until the symptoms resolve, and objective testing improves. In our experience, patients with symptomatic nyctalopia generally had normal objective test results 3–5 days afer stopping fenretinide.

Tamoxifen has been reported to decrease total cholesterol levels [23, 24], while fenretinide therapy has been reported to increase triglyceride levels in some patients [19]. Neither agent alone is known to significantly increase HDL levels. Tamoxifen treatment alone has been associated with small decreases in HDL levels [23, 25], but other studies have not confirmed this [26, 27]. It is reassuring that significant increases in triglyceride levels were not seen with tamoxifen and fenretinide in our study and that fenretinide did not seem to interfere with tamoxifen's salutory effects on total cholesterol levels. Our observation that this combination may increase HDL levels is intriguing in light of the interest in this regimen for breast cancer prevention. Larger studies are needed to further investigate this finding.

Tamoxifen and fenretinide therapy did not significantly increase total plasma TGF-β1 levels after one and three months of treatment. We were also unable to demonstrate any increase in plasma TGF-β2 or TGF-β3 levels with therapy. The plasma levels of these cytokines, therefore, do not appear to be modulated by tamoxifen and fenretinide and will not be useful as surrogate endpoint biomarkers. These findings differ from those of other investigators who have reported changes in plasma TGF-β1 [28] or TGF-β2 (29) levels which correlated with disease status or response to therapy. Differing patient populations and assay sensitivities may explain these discrepant results. Since elevated plasma TGF-β1 levels have been shown to be associated with pulmonary drug toxicity and veno-occlusive disease in breast cancer patients undergoing autologous bone marrow transplantation [30], it is reassuring that tamoxifen and fenretinide therapy did not notably increase serum levels of any of the TGF-β isoforms. What relation the serum TGF-β levels have, if any, to epithelial tissue levels is not known.

Adding fenretinide to tamoxifen as treatment for ER-positive patients who had been previously treated with tamoxifen appeared to benefit only one of 19 patients; no patient with ER-negative breast cancer benefited from this combination. None of the three evaluable ER-positive patients without prior hormonal therapy had an objective response although two of the three had stable disease for 8 months. However, in the previous Phase I/II study of this combination in 12 metastatic breast cancer patients who had not received prior treatment with tamoxifen, five patients had stable or improved disease lasting at least 6 months [10].

Although the tamoxifen and fenretinide combination does not appear to be obviously superior to tamoxifen alone as treatment for metastatic disease, the ongoing adjuvant and chemoprevention studies will help further elucidate the clinical utility of this regimen.

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